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The influence of oxygen concentration in liquid medium on elemental sulphur oxidation by *Thiobacillus thiooxidans*

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Abstract Rate of elemental sulphur biooxidation by *Thiobacillus thiooxidans* bacteria in continuous culture with nutrient circulation was determined for oxygen concentration in liquid in the range 1–17 mg/dm^3 for temperature range 19–40 °C, pH from 1.5 to 4.5 and for CO_2 concentration above 110 mg/dm^3. Equation describing the influence of above mentioned parameters on the rate of sulphur oxidation was presented.

List of symbols

C_{AB}	alive bacteria protein concentration, [μg/ml]
C_B	total bacteria protein concentration, [μg/ml]
C_{O_2}	oxygen concentration in liquid medium, [μg/dm^3]
C_1	total protein concentration in liquid, [μg/ml]
C_2	protein concentration in supernatant, [μg/ml]
R_{RBES}	rate of biooxidation of elemental sulphur, [mg S^0/ m^2* h)]
T	temperature, [°C]
X_B	alive bacteria fraction

1 Introduction

The *Thiobacillus thiooxidans* bacteria are commonly known as strictly aerobic however there doesn't exist any quantitative data showing the influence of oxygen concentration both in liquid nutrient and in gas over the nutrient on the rate of elemental sulphur oxidation caused by this bacteria. There are only few qualitative data according to which the rate increases with the increase of oxygen concentration in gas contacting with liquid nutrient in which elemental sulphur was suspended [1]. Positive effects of shaking the culture against the stationary cultures [2] as well as the increase of sulphur biooxidation rate caused by aeration of both stationary and shaking culture [3, 4] confirm the very important influence of oxygen transportation on the rate. The aim of this work was to determine quantitatively the influence of oxygen concentration in liquid medium on sulphur oxidation rate and *Thiobacillus thiooxidans* bacteria concentration.

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2 Materials and methods

2.1 Bacterial strain

Studies were performed using *Thiobacillus thiooxidans* strain originally isolated from the surface waters of sulphur ore mine Machów near Tarnobrzeg (Poland). As an inoculum eight days old stationary culture containing 10^6–10^7 cells/ml was used.

2.2 Culture medium

Modified Waksman mineral salts medium was used for cultivation. It consisted of: 2.0 g of $(NH_4)SO_4$, 0.5 g of KCl, 0.5 g of KH_2PO_4, 1.02 g of $MgSO_4 \cdot 7H2O$, 0.02 g of $FeSO_4$ $7H_2O$, 1 dm^3 of distilled water. pH of the medium was 4.5.

2.3 Neutralizing solution

To stabilize the pH of culture medium during the experiments the culture medium as in 2.2 was applied, but $(NH_4)_2SO_4$ was replaced with 2.39 g NH_4HCO_3. pH of the neutralizing solution was 8.5.

2.3.1 Procedure of determination of sulphur biooxidation rate

The rate of biooxidation of elemental sulphur (R_{RBES}) was defined as the amount of elemental sulphur oxidized per unit of time by the bacteria operating on a unit of elemental sulphur surface exposed to their action. Rate can be determined according to the method proposed earlier [5].

In this method RRBES is based on the sulphate ions' production rate. The rate of sulphate ions production was determined on the basis of SO_4^{2-} ions balance which were produced by the bacteria, introduced into the setup and led out of it. This rate was then recalibrated into rate of elemental sulphur oxidation (according to stoichiometric equation) and related to the size of elemental sulphur surface.

2.4 Bioreactor

As a bioreactor we used a glass cylinder containing a bed of sulphur pellets and inert glass pellets. The pellets were

arranged in circular layers looking from the bottom of the reactor: 4 layers of inert pellets, 1 layer of sulphur pellets, 7 layers of inert pellets. There were 65 to 68 sulphur pellets in the bioreactor during the experiments.

2.5
Experimental set-up

The main part of the setup, Fig. 1, was the bioreactor (1) containing the solid bed of sulphur and glass pellets as described above. Culture medium was pumped from the bioreactor through the rotameter and then to the bubble column where it was enriched in carbon dioxide and oxygen. From the bubble column the mixture of gas and liquid was turned back to the bioreactor.

The pH of the culture medium was controlled by the pH electrode ("CORNING") connected with the pH meter and the pH controller. When the pH dropped below the needed value, pH controller turned on the microdose pump that pumped in the nutrient solution and at the same time pumped out the same volume of the medium circulated in the setup.

The loss of culture medium due to taking out medium for analyses was compensated by adding sterile, fresh culture medium.

2.6
Sulphate ions determination

Sulphate ions were detected in all streams using liquid chromatograph HPLC ("WATERS") with conductometer detector, anion AR HR column and gluconic-borate solution as an eluent.

2.7
Determination of oxygen and carbon dioxide concentration in culture medium

Oxygen concentration in circulating medium was determined using ion selective electrode, "ORION RES.", model 97-08.

Carbon dioxide concentration in circulated stream was determined using ion selective electrode, "ORION RES." model 95-02.

2.8
Determination of alive bacteria protein concentration

Protein concentration was determined according to the Lowry's method [6] using bowin albumin as a standard.

Alive bacteria protein concentration was determined in the circulating culture medium according to a new, proposed method as follows: Liquid, taken from the setup, was divided into 2 parts. Each part was treated in a different way. First part was disintegrated using ultrasounds and after that the total protein concentration was determined (C_1). Second part was centrifuged (15,000 rpm for 20 min.) and after that protein concentration was determined in supernatant (C_2). The difference of protein concentration in the two parts ($C_B = C_1 - C_2$) was the bacteria *Thiobacillus thiooxidans* protein concentration. Then we estimated the fraction of alive bacteria (X_B) using epifluorescence microscopy (slides were dyed with acridine orange [7, 8]).

The protein concentration of alive bacteria we obtained by multiplying the bacterial protein concentration and the fraction of alive microorganisms ($C_{AB} = C_B \cdot X_B$).

3
Results

All experiments were performed for carbon dioxide concentration in liquid medium in the range of 110–150 mg/dm^3, in which the rate value is independent of CO_2 concentration as has been shown in previous paper [9].

Experiments were carried out at steady state conditions; during the experiments the values of pH, temperature, oxygen concentration remained constant in time. Their values were changed from experiment to experiment in the following way:

1. experiments for constant temperature (33 °C) performed with pH 1.5, 2.0, 3.0, 4.5,
2. experiments for constant pH (3.0) performed with temperatures 19 °C, 25 °C, 33 °C, 40 °C.

In all experiments oxygen concentration in liquid was changed by changing its concentration in gas used for liquid saturation with oxygen and carbon dioxide. Oxygen concentration in liquid equaled the concentration of saturation (Henry's law [10]) and was changed from 1 mg/dm^3 (approx 2 vol% in gas phase) to approx. 17 mg/dm^3 (approx. 53 vol% in gas phase). Low oxygen concentrations were obtained by gas dilution.

3.1
The influence of oxygen on sulphur oxidation by *Thiobacillus thiooxidans*

The influence of oxygen concentration in culture medium on the rate of sulphur oxidation by the *Thiobacillus thiooxidans* bacteria is presented in Fig. 2a–c. In all presented cases of constant pH and constant temperature an increase of sulphur biooxidation rate with the increase of oxygen concentration in culture medium was obtained.

The greatest oxidation rates we obtained in a culture with optimal conditions (pH-3.0, temperature 33 °C). To obtain in others cultures the same reaction rate as for optimal conditions we had to increase the oxygen concentration twice or three times depending on distance from optimal point. For example: in culture medium saturated with air (oxygen concentration in liquid was 7.37 mg/dm^3), for optimal conditions the sulphur biooxidation rate was 156.3 mg S^0/m^2*h. To obtain the same oxidation rate at pH-3.0 and temperature 25 °C it was necessary to increase the oxygen concentration to 9.5 mg/dm^3.

When the oxygen concentration reached 18 mg/dm^3 in culture at optimal conditions we obtained a sulphur biooxidation rate higher then 800 mg S^0/m^2*h. This shows the great possibility to increase the rate of the process by an increase of O_2 concentration in liquid.

3.2
The influence of oxygen concentration in culture medium on concentration of *Thiobacillus thiooxidans* bacteria

The influence of oxygen concentration in liquid medium on concentration of *Thiobacillus thiooxidans* bacteria is presented at Fig. 3a–c.

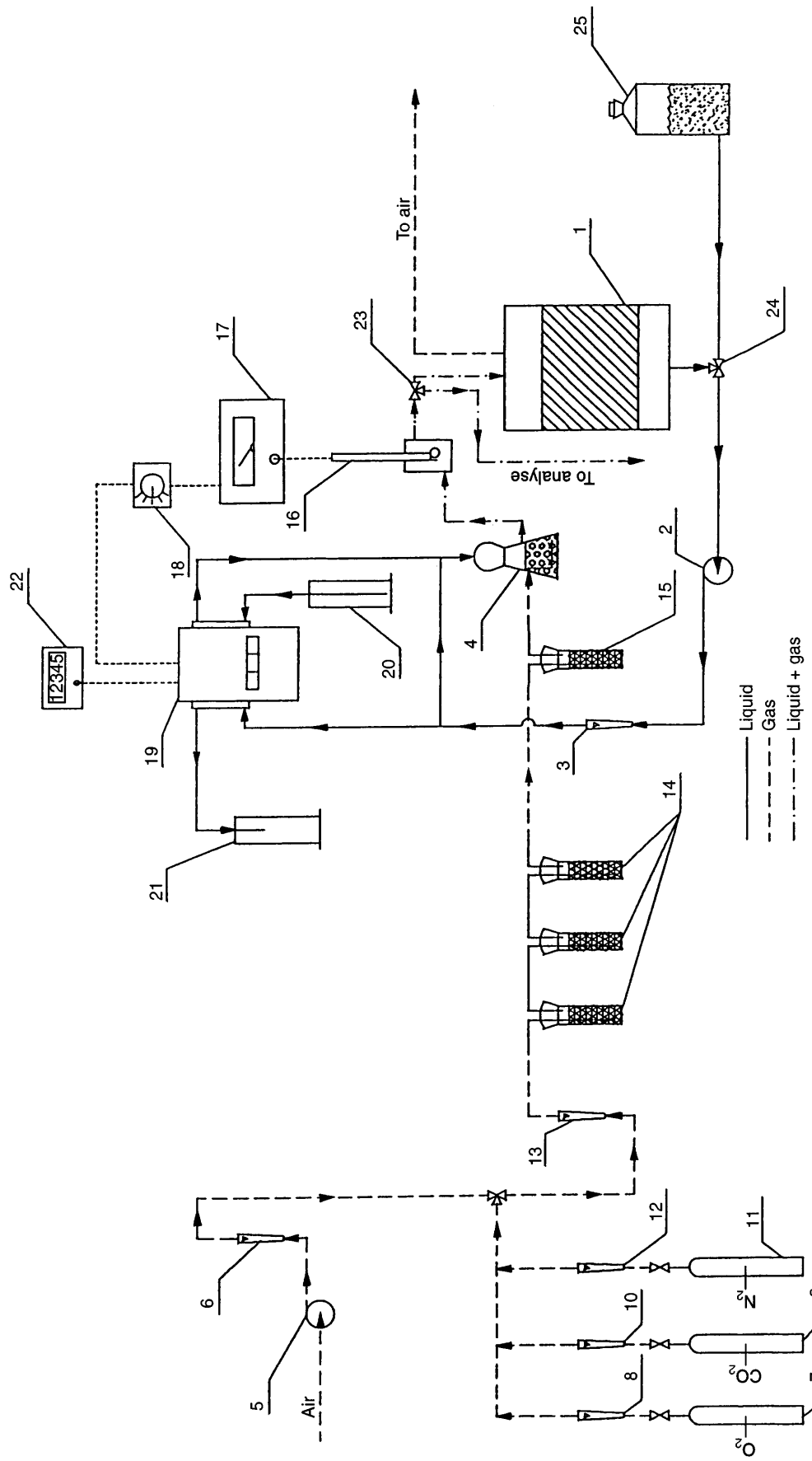

Fig. 1. Experimental set-up. *1* bioreactor, *2* peristaltic pump, *3* liquid rotameter, *4* barbotage washer, *5* membrane pump, *6, 8, 10, 12, 13* gas rotameters, *7* oxygen, *9* carbon dioxide, *11* nitrogen, *14* gas filters, *15* barbotage washer for gas humidification, *16* pH electrode, *17* pH meter, *18* pH regulator, *19* microdose pump, *20* bottle with neutralizer, *21* liquid collector, *22* impulse counter, *23* value for sampling, *24* valve for nutrient supply, *25* bottle with nutrient

Development of *Thiobacillus thiooxidans* bacteria in culture was measured as concentration of the alive bacteria protein concentration (see 2.8.) in the liquid circulating in the system. The process was investigated at steady-state condition so that equilibrium between the number of bacteria at the sulphur surface and the number of bacteria in liquid can be assumed [11].

In all presented cases an increase of bacterial protein concentration with increase of oxygen concentration in culture medium was observed. The maximum of protein

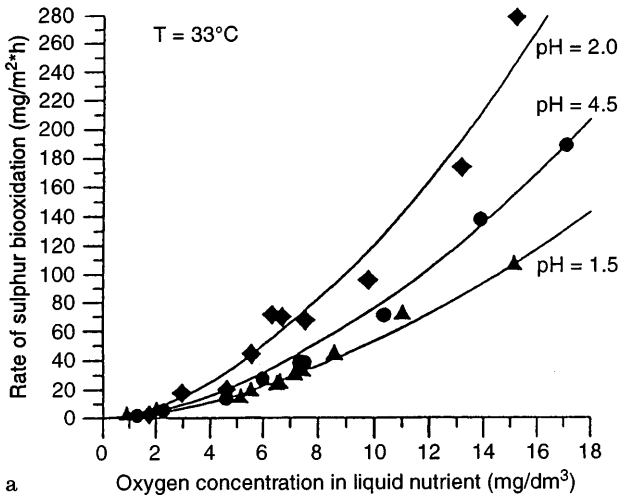

Fig. 2a–c. The influence of oxygen concentration in culture medium on the rate of sulphur oxidation by *Thiobacillus thiooxidans* bacteria at different pH and temperature

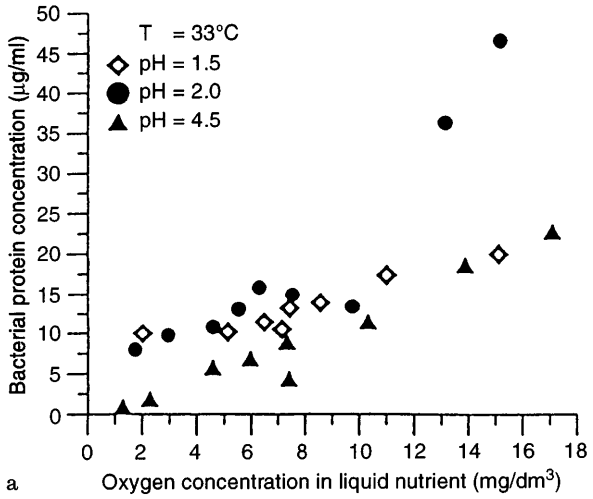

Fig. 3a–c. The influence of oxygen concentration in culture medium on the concentration of *Thiobacillus thiooxidans* bacteria in liquid at different pH and temperature

concentration was observed for optimal conditions (pH-3.0, temperature −33 °C). Increase of oxygen concentration from approximately 7.5 mg/dm^3 to approximately 10 mg/dm^3 gave an increase of protein concentration beyond 90 μg/ml. When the temperature or pH of nutrient differed from the optimal value then the increase of protein concentration with increase of oxygen concentration was much smaller. The temperature had greater influence on *Thiobacillus thiooxidans* bacteria protein concentration then pH of the medium.

4 Discussion

The experimental data presented in this paper show a very significant influence of oxygen concentration in liquid culture on the rate of sulphur oxidation by *Thiobacillus thiooxidans* bacteria. For constant pH in the range 1.5–4.5 and for constant temperature from 19° to 40° C as well as for carbon dioxide concentration in liquid higher than 110 mg/dm^3 (no impact of CO_2 on rate) the dependence rate-oxygen concentration can be described by a power function:

$$R_{\mathrm{RBES}} \doteq \text{constant } C_{\mathrm{O2}}^{1.7} \tag{1}$$

The value of the constant in this equation depends on temperature ($f(T)$) and on pH ($f(\mathrm{pH})$) as given by the following equation:

$$f(T) = 0.4282 - 0.04996 * T + 0.00193 * T^2 - 2.33 * 10^5 T^3 \tag{2}$$

$$f(\mathrm{pH}) = \exp[2.2342 * (\mathrm{pH}) + 0.04693 * (\mathrm{pH})^2 - 0.0819 * (\mathrm{pH})^3] \tag{3}$$

The equation for the rate of sulphur oxidation by *Thiobacillus thiooxidans* bacteria for different temperatures and different pH values within the investigated range can now be written as:

$$R_{\mathrm{RBES}} = f(T) * f(\mathrm{pH}) * C_{\mathrm{O_2}}^{1.7} \tag{4}$$

The mean standard deviation is 22, 32%.

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